United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 504

Supplemental Assay Method for the Manual Determination of Protein Content of Veterinary Biologics (Biuret)

Date: **March 19, 2008**

Number: SAM 504.04

Supersedes: TCSAM0504.03, April 4, 2005

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Supplemental Assay Method for the Manual Determination of Protein Content in Veterinary Biologics (Biuret)

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1. Introduction

This Supplemental Assay Method (SAM) describes the measurement of the protein content of various veterinary biologics products (serum, antiserum, and antitoxins) that is often utilized in the evaluation of such products. The following details the classical biuret procedure for the indirect determination of protein concentration (**Section 7**).

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

2.1.1 Spectrophotometer or colorimeter--Bausch and Lomb Spectronic 70 with cuvettes (or colorimeter with 1 cm or greater path length)

2.1.2 Common laboratory apparatus and glassware--pipettes, pipettors with tips, screw cap tubes, class A volumetric flasks, linear graph paper

2.1.3 Computer--with linear regression program (optional)

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

2.2.1 Phosphate buffered saline (PBS)--0.01M, pH 7.2-7.4 (National Veterinary Services Laboratories [NVSL] Media #30033), store at 4°C, stable for at least 6 months

2.2.2 Biuret reagent--(NVSL Media #10307), store at room temperature, stable for at least 6 months

Critical control point: Biuret reagent should be replaced when crystals or other precipitates appear in the solution.

2.2.3 Standard protein solution--Crystalline bovine albumin containing a known amount of protein

2.2.4 Bovine serum reference--Normal bovine serum (NVSL Media #40032, bovine serum [donor]), store at -20°C, stable at least 1 year

3. Preparation for the Test

3.1 Personnel qualifications/training

No specific training is required. Individuals should have working knowledge of laboratory equipment listed in **Section 2**.

3.2 Preparation of equipment/instrumentation

Turn on spectrophotometer to allow instrument to "warm up" for at least 30 minutes.

3.3 Preparation of reagents/control procedures

Reagents are prepared according to standard practice.

3.4 Preparation of the sample

Samples are normally sera, antisera or antitoxins, or serum fractions. Occasionally, biuret tests are run on other solutions, such as antigens. Follow sample receipt procedures as described by standard operating procedures

4. Performance of the Test

4.1 Standards

4.1.1 Standard solutions--Dilute Bovine Albumin with PBS to contain 10 mg/mL protein. Use 10 mg/mL solution as stock for curve. Dilute as listed below to establish a working standard curve.

CONC.(mg/mL)	ML STOCK	ML PBS
10	1.0	0
8	0.8	0.2
6	0.6	0.4
4	0.4	0.6
2	0.2	0.8
1	0.1	0.9

4.1.2 Run duplicate tubes of each solution (**Section 4.1.1**) using the test method (**Section 4.2**) to establish a standard curve. Plot average optical density (OD) for each point on graph paper (concentration vs. OD) or enter data into computer program to plot curve and calculate test results. If OD values for any point differ more than 0.05, disregard that point. If more than one point has unacceptable OD variations, rerun standard curve.

Critical control point: A standard curve is accurate for that lot of Biuret reagent. A comparison run or a new curve must be run when a new lot is used.

4.2 Test method

4.2.1 Dilute sample and bovine serum control 1:10 or 1:20 with PBS. Mix gently. (Sample dilution is based on the sample appearance or prior knowledge of sample. Dilute sample so OD falls on standard curve.)

4.2.2 Transfer 1 mL of dilution (**Section 4.2.1**) to tube or cuvette. Run duplicates.

4.2.3 Transfer 1 mL PBS to tube or cuvette for instrument blank.

4.2.4 Add 4 mL biuret reagent to each tube and mix gently. Let stand at room temperature for 30 to 45 minutes for color development.

4.2.5 Read OD at 540 nm, using blank to set zero. Read and record OD.

5. Interpretation of the Test Results

5.1 Calculation

Determine sample value (either read from curve and multiply x dilution or enter data into computer program). Average test results of duplicate tests. Test results are acceptable if the duplicate test results vary no more than 5% from the mean and the protein value for the bovine serum control falls within 5% of the established value.

5.2 Retest

If the OD of the diluted sample reads outside the end points on the standard curve, redilute sample and rerun the test.

6. Report of Test Results

Test results are reported following the current standard operating procedures.

7. References

7.1 Robinson, H. W. and Hogden, C. G. (1940) J. Biol. Chem., vol. 135, pp. 707-725.

7.2 Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) J. Biol. Chem., vol. 177, pp. 751-766.

7.3 Kibrick, A.C. (1958) Clin. Chem., vol. 4, pp. 232-236.

7.4 Kingsley, G. R. (1939) J. Biol. Chem., vol. 131, 1971.

8. Summary of Revisions

Version .04

- The document number has been changed from TCSAM0504 to SAM 504.

Version .03

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the name of the contact person has changed.

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Appendix I

NVSL Media #30033, PBS 0.01 M

Potassium phosphate, monobasic	0.34 g
Sodium phosphate dibasic, anhydrous	1.10 g
Sodium chloride	8.50 g
Sodium phosphate, dibasic	0.15 g

pH to 7.2-7.4.

Container: 1-L glass bottle

Method of sterilization: autoclave

Special instructions: Dissolve ingredients in 400-500 mL water and dilute to volume.

Appendix II

NVSL Media #10307, Biuret Reagent

Copper sulfate, pentahydrate (dissolve in 400 mL water)	1.50 g
Potassium sodium tartrate (add to above and dissolve)	6.00 g
Sodium hydroxide (dissolve in separate 300 mL water, add to above)	30.0 g
Potassium iodide (add to above after other chemicals are dissolved and dilute to 1 L)	1.00 g

Container: 1-L glass bottle

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Appendix III

NVSL Media #40032, Bovine serum--not sterilized

Container: 5-mL serum vial

Special instructions: Donor bovine serum, mix thoroughly, dispense 3-mL into serum vials.

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Quick Reference

_____Accession number and section number assigned

_____Submission paperwork correct

_____Adequate amount of sample(s)

_____Biuret (date prepared)

_____Sample(s) OD within standard curve limits

_____Results reviewed

_____Report generated

_____Report reviewed, signed, and sent